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(54) Title: PEPTIDE COMPOUNDS WHICH INHIBIT METALLOPROTEINASE AND TNF LIBERATION, AND THEIR THERAPEUTIC USE			
(57) Abstract			
Peptidyl compounds have therapeutic utility via MMP/TNF inhibition.			

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Peptide compounds which inhibit metalloproteinase and TNF liberation, and their therapeutic use

Field of the Invention

This invention relates to a novel class of peptidyl derivatives, to processes for their preparation, and to their use in medicine.

5 Background to the Invention

Tumour Necrosis Factor (TNF) is a cytokine which is produced initially as a cell-associated 28kD precursor. It is released as an active, 17kD form (D-M Jue *et al*, (1990) *Biochemistry*, 29:8371-8377), which can mediate a large number of deleterious effects *in vivo*. When administered to animals or humans it causes
10 inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase responses, similar to those seen during acute infections and shock states. Chronic administration can also cause cachexia and anorexia. Accumulation of excessive TNF can be lethal.

There is considerable evidence from animal model studies that blocking the
15 effects of TNF with specific antibodies can be beneficial in acute infections, shock states, graft versus host reactions and autoimmune disease. TNF is also an autocrine growth factor for some myelomas and lymphomas and can act to inhibit normal haematopoiesis in patients with these tumours.

Preventing the production or action of TNF is, therefore, predicted to be a
20 potent therapeutic strategy for many inflammatory, infectious, immunological or malignant diseases. These include, but are not restricted to, septic shock, haemodynamic shock and sepsis syndrome (Mathison *et al*, (1988) *J. Clin. Invest.* 81:1925-1937; Miethke *et al* (1992), *J. Exp. Med.* 175:91-98), post ischaemic reperfusion injury, malaria (Grau *et al* (1989), *Immunol. Rev.* 112:49-70);
25 mycobacterial infection (Barnes *et al* (1992) *Infect. Imm.* 60:1441-6), meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, cancer, autoimmune disease, rheumatoid arthritis, multiple sclerosis, radiation damage, toxicity following administration of immunosuppressive monoclonal antibodies such as OKT3 or CAMPATH-1 and hyperoxic alveolar injury.

30 Current clinical anti-TNF strategies involve the use of corticosteroids such as dexamethasone, and the use of cyclosporin-A or FK506, which are non-specific inhibitors of cytokine gene transcription. Phosphodiesterase inhibitors such as

pentoxifylline have been shown to be more specific inhibitors of TNF gene transcription (Endres S . (1991) *Immunol.* 72:56-60, Schandene *et al* (1992), *Immunol.* 76:30-34, Alegre ML, *et al* (1991); Transplantation 52:674-679, Bianco *et al* (1991) *Blood* 78:1205-1221). Thalidomide has also been shown to inhibit TNF
5 production by leucocytes (Sampajo *et al* (1991), *J. Exp. Med.* 173:699-703). In experimental settings, anti-TNF monoclonal antibodies, soluble TNF receptors and soluble TNF receptor/immunoadhesins have been shown to specifically inhibit the effects of TNF action (Bagby *et al* (1991) *J. Infect. Dis.* 163:83-88, Charpentier *et al.* (1991) *Presse-med.* 20:2009-2011, Silva *et al* (1990) *J. Infect. Dis.* 162:421-427;
10 Franks *et al* (1991) *Infect. Immun.* 59:2609-2614, Tracey *et al* (1987) *Nature* 330:662-664; Fischer *et al* (1992) *PNAS USA* in press, Lesslauer *et al* (1991) *Eur. J. Immunol.* 21:2883-2886, Ashkenazi *et al* (1991) *PNAS USA* 88:10535-10539).

It has recently been shown that the effects of TNF are mediated by two peptides, $\text{TNF}\alpha$ and $\text{TNF}\beta$. Although these peptides have only 30% homology with
15 each other, they activate the same receptors and are encoded by immediately adjacent genes. As used herein, the term tumour necrosis factor or TNF therefore means tumour necrosis factor α and peptides having a high degrees of sequence homology with, or substantially similar physiological effects to, $\text{TNF}\alpha$, for example $\text{TNF}\beta$.

One of the objectives of the present invention is to provide compounds which
20 substantially inhibit the release of TNF from cells, and therefore may be used in the treatment of conditions mediated by TNF. Such uses include, but are not limited to, the treatment of inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease.

25 In normal tissues, cellular connective tissue synthesis is offset by extracellular matrix degradation, the two opposing effects existing in dynamic equilibrium. Degradation of the matrix is brought about by the action of proteinases released from resident connective tissue cells and invading inflammatory cells, and is due, in part, to the activity of at least four groups of metalloproteinases. These are the
30 collagenases (interstitial collagenase, MMP-1; PMN collagenase, MMP-8, collagenase-3, MMP-13), the gelatinases (gelatinase A, MMP-2, 72kDa-gelatinase, Type IV collagenase; gelatinase B, MMP-9, 92kDa-gelatinase, Type IV collagenase)

the stromelysins (proteoglycanase, MMP-3, stromelysin-1, transin; stromelysin-2, MMP-10; stromelysin 3, MMP-11) and the membrane type matrix metalloproteinases (MT-1, MMP-14; MT-2, MMP-15; MT-3, MMP-16 and MT-4, MMP-17). Normally these catabolic enzymes are tightly regulated at the level of their synthesis and secretion and also at the level of their extracellular activity, the latter through the action of specific inhibitors, such as TIMP (tissue inhibitors of metalloproteinase), which form inactive complexes with metalloproteinases, and more general proteinase inhibitors such as α_2 -macroglobulins.

The accelerated, uncontrolled breakdown of connective tissues by metalloproteinase catalysed resorption of the extracellular matrix is a feature of many pathological conditions such as rheumatoid arthritis, osteoarthritis, septic arthritis, corneal, epidermal or gastric ulceration; tumour metastasis or invasion; periodontal disease, proteinuria, coronary thrombosis associated with atherosclerotic plaque rupture and bone disease. Inhibitors may also be useful in preventing the pathological sequelae following a traumatic injury that could lead to a permanent disability. These compounds may also have utility as a means for birth control by preventing ovulation or implantation. It can be expected that the pathogenesis of such diseases is likely to be modified in a beneficial manner by the administration of metalloproteinase inhibitors and numerous compounds have been suggested for this purpose [for a general review see R C Wahl, *et al* Ann. Rep. Med. Chem. 25, 175-184, Academic Press Inc., San Diego (1990)].

Compounds which have the property of inhibiting the action of metalloproteinases involved in connective tissue breakdown such as collagenase, stromelysin and gelatinase have been shown to inhibit the release of TNF both *in vitro* and *in vivo* (AJH Gearing *et al* (1994), Nature, 370:555-557; GM McGeehan *et al* (1994), Nature, 370:558-561; MJ Crimmin *et al*, WO 93/20047 and WO 94/10990). All of these reported inhibitors contain a hydroxamic acid zinc binding group.

It is, therefore, a further objective of this invention to provide compounds which, in addition to inhibiting TNF release, also may inhibit the action of certain MMPs, and hence may be used in the treatment of patients who suffer from conditions mediated by TNF and/or MMPs such uses include, but are not limited to

inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease; and those involving tissue breakdown such as bone resorption, inflammatory diseases, dermatological conditions, tumour growth, angiogenesis and invasion by secondary metastases, in particular rheumatoid arthritis, osteoarthritis, periodontitis, gingivitis, corneal ulceration, tumour growth, angiogenesis and invasion by secondary metastases.

Compounds that inhibit collagenase, which possess structural portions akin to those of the instant invention include those encompassed by U.S. 4,511,504 issued Apr. 16, 1985; U.S. 4,568,666, issued Feb 4, 1986.

Compounds of related structure that are claimed to inhibit stromelysin (proteoglycanase) are encompassed by U.S. 4,771,037, issued Sept. 13, 1988.

The applicants believe that stromelysin and collagenase inhibitors have utility in preventing articular cartilage damage associated with septic arthritis. Bacterial infections of the joints can elicit an inflammatory response that may then be perpetuated beyond what is needed for removal of the infective agent resulting in permanent damage to structural components. Bacterial agents have been used in animal models to elicit an arthritic response with the appearance of proteolytic activities. See J. P. Case *et al* (1989), *J. Clin. Invest.*, 84:1731-40; R. J. Williams *et al* (1990), *Arth. Rheum.*, 33: 533-41.

The applicants also believe that inhibitors of stromelysin, collagenase and gelatinase will be useful to control tumour metastasis, optionally in combination with current chemotherapy and/or radiation. See L. M. Matrisian *et al* (1986), *Proc. Natl. Acad. Sci., USA*, 83:9413-7; S. M. Wilhelm *et al* (1987), *Ibid.* 84:6725-29; Z. Werb *et al* (1989), *J. Cell Biol.*, 109:872-889; L. A. Liotta *et al* (1983), *Lab. Invest.*, 49:636-649; R. Reich *et al* in *Metastasis*; Ciba Foundation Symposium, Wiley, Chicester, 1988, pp. 193-210.

Secreted proteinases such as stromelysin, collagenase and gelatinase play an important role in processes involved in the movement of cells during metastatic tumour invasion. Indeed, there is also evidence that the matrix metalloproteinases are over expressed in certain metastatic tumour cell lines. In this context, the enzyme functions to penetrate underlying basement membranes and allow the tumour

cell to escape from the site of primary tumour formation and enter the circulation. After adhering to blood vessel walls, the tumour cells use these same metalloproteinases to pierce underlying basement membranes and penetrate other tissues, thereby leading to tumour metastasis. Inhibition of this process would
5 prevent metastasis and improve the efficacy of current treatments with chemotherapeutics and/or radiation.

These inhibitors should also be useful for controlling periodontal diseases, such as gingivitis. Both collagenase and stromelysin activities have been isolated from fibroblasts derived from inflamed gingiva (V. J. Uitto *et al* (1981),
10 J. Periodontal Res., 16:417-424). Enzyme levels have been correlated to the severity of gum disease; C. M. Overall *et al* (1987), J. Periodontal Res., 22:81-88.

Proteolytic processes have also been observed in the ulceration of the cornea following alkali burns (S. I. Brown *et al* (1969), Arch. Ophthalmol., 81:370-373). Mercapto-containing peptides do inhibit the collagenase isolated from alkali-burned
15 rabbit cornea (F. R. Burns *et al* (1989), Invest. Ophthalmol, 30:1569-1575). Treatment of alkali-burned eyes or eyes exhibiting corneal ulceration as a result of infection with inhibitors of these metalloendoproteinases in combination with sodium citrate or sodium ascorbate and/or antimicrobials may be effective in preventing developing corneal degradation.

20 Stromelysin has been implicated in the degradation of structural components of the glomerular basement membrane (GBM) of the kidney, the major function of which is to restrict passage of plasma proteins into the urine (W. H. Baricos *et al* (1989), Biochem. J., 254:609-612). Proteinuria, a result of glomerular disease, is excess protein in the urine caused by increased permeability of the GBM to plasma
25 proteins. The underlying causes of the increased GBM permeability are unknown, but proteinases including stromelysin may play an important role in glomerular diseases. Inhibition of this enzyme may alleviate the proteinura associated with kidney malfunction.

It is suggested that inhibition of matrix metalloproteinase activity may prevent
30 the rupturing of atherosclerotic plaques leading to coronary thrombosis. The tearing or rupture of atherosclerotic plaques is the most common event initiating coronary thrombosis. Destabilisation and degradation of the connective tissue matrix

surrounding these plaques by proteolytic enzymes or cytokines released by infiltrating inflammatory cells has been proposed as a cause of plaque fissuring. Such tearing of these plaques can cause an acute thrombolytic event as blood rapidly flows out of the blood vessel. High levels of stromelysin RNA message have been found to be localised to individual cells in atherosclerotic plaques removed from heart transplant patients at the time of surgery (A. M. Henney *et al* (1991), Proc. Nat'l. Acad. Sci. USA, 88:8154-8158). Inhibition of matrix metalloproteinases by these compounds may aid in preventing or delaying the degradation of the connective tissue matrix that stabilises the atherosclerotic plaques, thereby preventing events leading to acute coronary thrombosis.

It has been recently shown in a model of congestive heart failure (CHF) in the pig, that during CHF there are marked changes in the morphological structure of the heart. Ventricular dilation and wall thinning caused by changes to the extracellular matrix results in fewer collagen connections between cardiomyocytes and less total collagen. In such an instance a weaker force of contraction leads to an inefficient ventricular operation. It is believed that specific inhibitors of matrix metalloproteinases will play a key role in stabilising the extracellular matrix and therefore be important in the treatment and/or prevention of CHF.

It has recently been shown (WO 96/0240) that inhibitors of the matrix metalloproteinases, such as collagenase and stromelysin also inhibit the formation of human soluble CD23. CD23 is a 45kDa type II integral protein expressed on the surface of a variety of mature cells, including B and T lymphocytes, macrophages, NK cells, Langerhans cells, monocytes, eosinophils and platelets (Delespesse *et al*, Adv. Immunology, 49, 1991, 149; Grangette *et al*, J., Immunol, 143, 1989, 3580). Several activities have been ascribed to soluble CD23 in man, all of which involve IgE regulation. Particular activities include:

- i) antigen presentation
- ii) IgE mediated eosinophil cytotoxicity
- iii) B cell homing to lymph nodes and the spleen
- iv) downregulation of IgE synthesis

Thus, overall the excessive production of soluble CD23 has been implicated in the overproduction of IgE, the hallmark of allergic diseases such as extrinsic

asthma, rhinitis, allergic conjunctivitis, eczema, atopic dermatitis and anaphylaxis (Sutton et al, Nature, 366, 1993, 421). Elevated levels of soluble CD23 have also been observed in the serum of patients with chronic B lymphocytic leukaemia (Safarti *et al*, Blood, 71, 1988, 94), and in the synovial fluid of patients with
5 rheumatoid arthritis (Chomarat *et al*, Arthritis and Rheumatism, 36, 1993, 234).

It is therefore, a further objective of the present invention to provide compounds which inhibit the formation of human soluble CD23 for the production of a medicament for the treatment or prophylaxis of disorders such as allergy and autoimmune disease in which the overproduction of soluble CD23 is implicated, such
10 as those described above.

Recent reports suggest that new enzymes of the MMP family also mediate the shedding of adhesion molecules such as the selectins, such as L-selectin. These soluble adhesion molecules are implicated in a number of diseases including cancer, autoimmunity and in the inflammatory response. It has been proposed that once
15 cleaved, the selectin bind to particular ligands and this accounts for their biological activity. Thus, drugs that interfere with or prevent binding of the ligands to the selectins will be useful medicaments for treating a variety of the diseases described above. Therefore, it is a yet further objective of the present invention to provide compounds which inhibit the shedding of certain adhesion molecules and thus
20 provide the production of a medicament for the treatment or prophylaxis of disorders such as cancer, autoimmune diseases or inflammatory diseases (such as inflammatory bowel disease and multiple sclerosis).

It is also believed that specific inhibitors of stromelysin and collagenase should be useful as birth control agents. There is evidence that expression of metalloproteinases, including stromelysin and collagenase, is observed in unfertilised
25 eggs and zygotes and at further cleavage stages and increased at the blastocyst stage of fetal development and with endoderm differentiation (C. A. Brenner *et al* (1989), Genes & Develop., 3:848-59). By analogy to tumour invasion, a blastocyst may express metalloproteinases in order to penetrate the extracellular matrix of the uterine
30 wall during implantation. Inhibition of stromelysin and collagenase during these early development processes should presumably prevent normal embryonic development and/or implantation in the uterus. Such intervention would constitute

a novel method of birth control. In addition there is evidence that collagenase is important in ovulation processes. In this example, a covering of collagen over the apical region of the follicle must be penetrated in order for the ovum to escape. Collagenase has been detected during this process and an inhibitor has been shown
5 to be effective in preventing ovulation (J. F. Woessner *et al* (1989), *Steroids*, 54:491-499). There may also be a role for stromelysin activity during ovulation (C. K. L. Too *et al* (1984), *Endocrin.*, 115:1043-1050).

Collagenolytic and stromelysin activity have also been observed in dystrophic epidermolysis bullosa (A. Kronberger *et al* (1982), *J. Invest. Dermatol.*, 79:208-211;
10 D. Sawamura *et al* (1991), *Biochem. Biophys. Res. Commun.*, 184:1003-8). Inhibition of metalloendoproteinases should limit the rapid destruction of connective components of the skin.

In addition to extracellular matrix comprising structural components, stromelysin can degrade other *in vivo* substrates including the inhibitors α_1 -proteinase
15 inhibitor and may therefore influence the activities of other proteinases such as elastase (P. G. Winyard *et al* (1991), *FEBS Letts.*, 279:1:91-94). Inhibition of the matrix metalloendoproteinases may potentiate the antiproteinase activity of these endogenous inhibitors.

From recent publications it is evident that several new enzymes of the MMP
20 family have been identified, some of which maybe important in disease. Collagenase 3, an enzyme found in to breast carcinoma tissue and other disease states such as arthritis, may have utility in breast cancer (JMP Freije *et al* (1994), *J. Biol. Chem.*, 269 (24): 16766-16773), whilst MT-MMPs, other members of the MMP family have been shown to be key enzymes in the activation of gelatinase A (H Sato *et al* (1994),
25 *Nature*, 370:61-65). Gelatinase A is an important enzyme in the growth and metastasis of tumours (such as defined above).

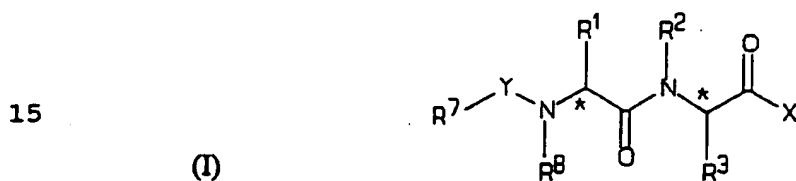
The degradation of β -Amyloid Precursor Protein (APP) has been shown to generate amyloid plaques, a major constituent of the senile plaques, found in patients with Alzheimers Disease (AD). Two recent publications have identified
30 metalloproteinase enzymes that cleave APP to the amyloid plaque (CR Abraham *et al* (1994), *Biochemistry*, 33:192-199; G Huber *et al* (1994), *Biochem. Biophys. Res. Comm.*, 201 (1):45-53).

As appreciated by those of skill in the art, the significant proportion of homology between these new enzymes and other MMPs leads to the possibility that a compound that inhibits one enzyme may to some degree inhibit these new enzymes. Therefore, inhibitors encompassed in this invention may be useful in the diseases in which these new enzymes are implicated.

SUMMARY OF THE INVENTION

The invention encompasses novel compounds of formula (I) which are useful inhibitors of matrix metalloproteinase and/or TNF mediated diseases including degenerative diseases (such as defined above) and certain cancers.

In a first aspect of the invention there is provided a compound of general formula (I)



20

Wherein:

Y is C=O, C=S or S(O)_n, where n is 1 or 2;

R¹ is a C₁₋₆ alkyl, C₂₋₆ alkenyl, C₁₋₆ alkyl-aryl, aryl, C₁₋₆ alkyl-heteroaryl, heteroaryl or C₁₋₆ alkyl-AR⁹ group where A is O, NR⁹ or S(O)_m where m=0-2, and R⁹ is H, C₁₋₄ alkyl, aryl, heteroaryl, C₁₋₄ alkyl-aryl or C₁₋₄ alkyl-heteroaryl. If A=NR⁹ the R⁹ groups may be the same or different.

R² is hydrogen or a C₁₋₆ alkyl group;

R³ is a [Alk]_nR⁶ group where Alk is a C₁₋₆ alkyl or C₂₋₆ alkenyl group and n is zero or an integer 1;

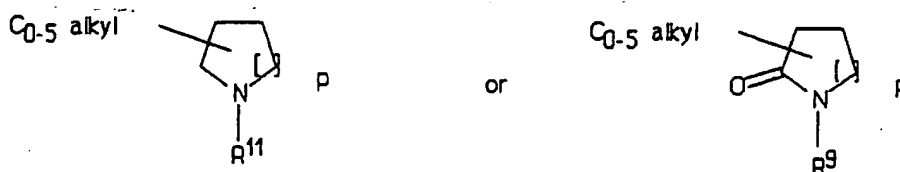
X is a group NR⁴R⁵ where R⁴ is hydrogen or an aryl, heteroaryl, C₁₋₆ alkyl-heteroaryl, cyclo(C₃₋₆) alkyl, C₁₋₆ alkyl-cyclo(C₃₋₆)alkyl, heterocyclo(C₄₋₆)alkyl (such as pyrrolidine or piperidine) or C₁₋₆ alkyl-heterocyclo(C₄₋₆)alkyl group or the group

10

C_{1-6} alkyl optionally substituted by amino (NH_2), aryl, arylamino, protected amino, di(C_{1-6} alkyl)amino, mono(C_{1-6} alkyl)amino, CO_2H , protected carboxyl, carbamoyl, mono(C_{1-6} alkyl) carbamoyl, di(C_{1-6} alkyl) carbamoyl, and R^5 is hydrogen; NR^4R^5 may also form a ring such as pyrrolidino, piperidino or morpholino group.

- 5 R^7 is aryl (optionally substituted with R^{10}), heteroaryl (optionally substituted with R^{10}), C_{1-5} alkyl (optionally substituted with R^{10}), C_{1-5} alkyl-aryl (optionally substituted with R^{10}), C_{1-5} alkyl-heteroaryl (optionally substituted with R^{10}), cyclo (C_{3-6}) alkyl (optionally substituted with R^{10}), cyclo (C_{3-6}) alkenyl (optionally substituted with R^{10}), C_{1-5} alkyl-cyclo (C_{3-6}) alkyl (optionally substituted with R^{10}), the groups

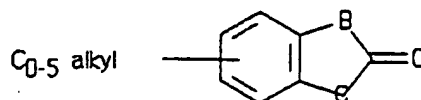
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where $p=1-2$, or the group

20



- 25 where B and C may be selected from the groups O, S, $C(R^9)_2$, or NR^9 and these may be the same or different;

When Y is $C=O$, R^7 can also be CO_2R^2 ;

R^8 is hydrogen;

R^6 is an optionally substituted cyclo (C_{3-6}) alkyl, cyclo (C_{3-6}) alkenyl, C_{1-6} alkyl, aryl, C_{1-6} alkoxy-aryl, benzyloxyaryl, heteroaryl, COR^9 , C_{1-3} alkyl-aryl, C_{1-3} alkyl-heteroaryl, C_{1-6} alkyl- CO_2R^9 , C_{1-6} alkyl- NHR^9 , AR^9 , CO_2R^2 , $CONHR^{12}$, $NHCOR^{12}$,
 5 $NHCO_2R^{12}$, $NHSO_2R^{12}$, amidine or guanidine group;

where R^{12} is a group C_{1-4} alkyl, C_{1-4} alkylaryl, C_{1-4} alkyl-heteroaryl, cyclo (C_{3-6}) alkyl, C_{1-4} alkyl-cyclo (C_{3-6}) alkyl, C_{2-6} alkenyl, C_{2-6} alkenyl-aryl, aryl or heteroaryl as defined above.

R^{10} is AR^9 , COR^{13} , SO_2R^9 (where R^9 is not H), $SO_2N(R^9)_2$, NR^9R^{11} , COR^9 ,
 10 phthalimido or succinimido;

R^{11} is a COR^9 , CO_2R^2 (where R^2 is not H), $CONHR^9$ or SO_2R^9 (where R^9 is not H) group;

R^{13} is a OH, OC_{1-4} alkyl, $O(C_{1-4}$ alkyl) aryl, $N(R^9)_2$ (in which R^9 is the same or different);

15 and the salts, solvates and hydrates thereof.

Preferred compounds of the invention include those in which, independently or in any combination have:

Y is $C=O$, $C=S$ or SO_2 ;

R^1 is C_{1-6} alkyl or C_{1-4} alkyl- AR^9 where A is $S(O)_m$, NR^9 , or O and $m=0,1$ or 2,
 20 and R^9 is H, C_{1-4} alkyl heteroaryl or aryl;

R^2 is H or C_{1-4} alkyl;

R^3 is $[Alk]_nR^6$ where $n=0$ or 1, Alk is C_{1-4} alkyl and R^6 is C_{1-4} alkyl, C_{1-3} alkyl-aryl, C_{1-3} alkyl-heteroaryl, AR^9 , CO_2R^2 , $CONHR^{12}$, $NHCOR^{12}$, $NHCO_2R^{12}$, $NHSO_2R^{12}$,
 amidine or guanidine group;

25 R^5 is H;

R^4 is H, C_{1-6} alkyl, aryl, heteroaryl, C_{1-2} alkyl-aryl or C_{1-2} alkyl-heteroaryl;

NR^4R^5 may form a 5-7 membered ring such as a pyrrolidine, piperidine or morpholine;

R^7 is aryl (optionally substituted with R^{10}), heteroaryl (optionally substituted with R^{10}), C_{1-4} alkyl-aryl (optionally substituted with R^{10}), C_{1-4} alkyl-heteroaryl (optionally substituted with R^{10}), C_{1-5} alkyl- R^{10} , C_{1-5} alkenyl- R^{10} , cyclo(C_{3-6})alkyl- R^{10} ; when Y is $C=O$, R^7 can also be CO_2R^2 ;

30

R⁸ is hydrogen;

R¹⁰ is COR¹³, NR⁹R¹¹, N(R⁹)₂, phthalimido or succinimido;

R¹¹ is COR⁹, CO₂R⁹ (provided R⁹ is not H), or SO₂R⁹ (provided R⁹ is not H);

R¹² is C₁₋₄ alkyl, C₁₋₄ alkylaryl, C₁₋₄ alkyl-heteroaryl;

5 and R¹³ is OH, OC₁₋₄ alkyl or N(R⁹)₂;

Compounds of the invention have IC₅₀ values below 50mM against the MMP enzymes and/or below 50mM in the whole cell assay of TNF inhibition.

It will be appreciated that the compounds according to the invention can contain one or more asymmetrically substituted carbon atoms, for example those
10 marked with an asterisk in formula (1). The presence of one or more of these asymmetric centres in a compound of formula (1) can give rise to stereoisomers, and in each case the invention is to be understood to extend to all such stereoisomers, including enantiomers and diastereomers, and mixtures including racemic mixtures thereof.

15 In the formulae herein, the ~ line is used at a potential asymmetric centre to represent the possibility of *R*- and *S*- configurations, the < line and the line to represent a unique configuration at an asymmetric centre.

As used in this specification, alone or in combination, the term "C₁₋₆ alkyl" refers to a straight or branched chain alkyl moiety having from one to six carbon
20 atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, *tert*-butyl, pentyl, hexyl and the like.

The term "C₁₋₄ alkyl" refers to a straight or branched chain alkyl moiety having from one to four carbon atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, *tert*-butyl and the like.

25 The term "C₂₋₆ alkenyl" refers to a straight or branched chain alkyl moiety having two to six carbon atoms and having in addition one double bond, of either *E* or *Z* stereochemistry where applicable. This term would include for example, vinyl, 1-propenyl, 1- and 2-butenyl, 2-methyl-2-propenyl etc.

The term "cyclo(C₃₋₆)alkyl" refers to a saturated alicyclic moiety having from
30 three to six carbon atoms and includes for example cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

The term "cyclo(C₃₋₆)alkenyl" refers to an alicyclic moiety having from three to six carbon atoms and having in addition one double bond. This term would include for example cyclopentenyl or cyclohexenyl.

5 The term "heterocyclo(C₄₋₆)alkyl" refers to a saturated heterocyclic moiety having from three to six carbon atoms and one or more heteroatom from the group N, O, S and includes for example azetidiny, pyrrolidinyl, tetrahydrofuranyl, piperidinyl and the like.

There term "aryl" means an optionally substituted phenyl or naphthyl group with the substituent(s) being selected, for example, from halogen, trifluoromethyl, 10 C₁₋₆ alkyl, alkoxy, phenyl and the like.

The term "halogen" means fluorine, chlorine, bromine or iodine.

The terms "protected amino" and "protected carboxy" mean amino and carboxy groups which are protected in a manner familiar to those skilled in the art. For example, an amino group can be protected by a benzyloxycarbonyl, *tert*-butoxycarbonyl, acetyl or like groups, or in the form of a phthalimido or like group. 15 A carboxyl group can be protected in the form of a readily cleavable ester such as the methyl, ethyl, benzyl or *tert*-butyl ester.

The term "alkoxy" refers to a straight chain or branched chain alkoxy group containing a maximum of six carbon atoms, such as methoxy, ethoxy, propoxy, 20 isopropoxy, butoxy, *tert*-butoxy and the like.

The term "C₁₋₅ alkyl" refers to a straight chain or branched chain alkoxy group containing a maximum of five carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, *tert*-butyl, pentyl and the like.

The term "heteroaryl" refers to aromatic ring systems of five to ten atoms or 25 which at least one atom is selected from the group, O, N, or S and includes for example furanyl, thiophenyl, pyridyl, indolyl, quinolyl and the like.

The term "optionally substituted" refers to a group unsubstituted or substituted with a group taken from but not limited to halogen, trifluoromethyl, C₁₋₆ alkyl, phenyl or a group R¹⁰, where R¹⁰ has been defined previously, and the like.

30 Salts of compounds of formula (I) include pharmaceutically acceptable salts, for example acid addition salts derived from inorganic or organic acids, such as hydrochlorides, hydrobromides, p-toluenesulphonates, phosphates, sulphates, perchlorates, acetates, trifluoroacetates, propionates, citrates, malonates, succinates, lactates, oxalates, tartrates and benzoates.

Salts may also be formed with bases. Such salts include salts derived from inorganic or organic bases, for example alkali metal salts such as magnesium or calcium salts, and organic amine salts such as morpholine, piperidine, dimethylamine or diethylamine salts.

5 When the "protected carboxy" group in compounds of the invention is an esterified carboxyl group, it may be a metabolically labile ester of formula CO_2R^{14} where R^{14} may be an ethyl, benzyl, phenethyl, phenylpropyl, α or β -naphthyl, 2,4-dimethylphenyl, 4-*tert*-butylphenyl, 2,2,2-trifluoroethyl, 1-(benzyloxy)benzyl, 1-(benzyloxy)ethyl, 2-methyl-1-propionyloxypentyl, 2,4,6-trimethylbenzyloxymethyl or pivaloyloxymethyl group.

10 Compounds of the general formula (I) may be prepared by any suitable method known in the art and/or by the following processes, which itself forms part of the invention.

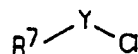
 According to a second aspect of the invention, there is provided a process for
15 preparing a compound of general formula (I) as defined above. It will be appreciated that where a particular stereoisomer of formula (I) is required, the synthetic processes described herein may be used with the appropriate homochiral starting material and/or isomers maybe resolved from mixtures using conventional separation techniques (e.g. HPLC).

20 The compounds according to the invention may be prepared by the following process. In the description and formulae below the groups R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} A, B, C and X are as defined above, except where otherwise indicated. It will be appreciated that functional groups, such as amino, hydroxyl or carboxyl groups, present in the various compounds described below, and
25 which it is desired to retain, may need to be in protected form before any reaction is initiated. In such instances, removal of the protecting group may be the final step in a particular reaction. Suitable protecting groups for such functionality will be apparent to those skilled in the art. For specific details see "Protective Groups in Organic Synthesis", Wiley Interscience, T W Greene, PGM Wuts.

30 Thus the process required for preparing compounds of general formula (I) comprises of:
reaction of an acid chloride of formula (II)

15

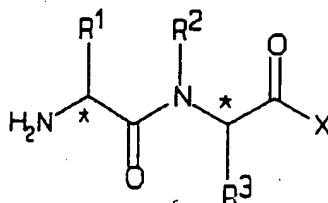
(II)



5 Wherein Y is C=O or SO₂ and R⁷ is as defined above, with an amine of formula (III)

10

(III)



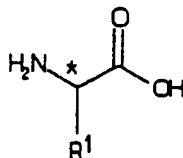
15

It will be appreciated that where a particular stereoisomer or formula (I) is required, this may be obtained by conventional resolution techniques such as high performance liquid chromatography. Where desired, however, appropriate homochiral starting materials may be used in the coupling reaction to yield a particular stereoisomer of formula (I). This is exemplified below.

The sulphonylation reaction may be performed using standard conditions for amination reactions of this type. Thus, the reaction may be achieved in a solvent, for example an inert organic solvent such as an ether, eg. a cyclic ether such as tetrahydrofuran, an amide eg. a substituted amide such as dimethylformamide, or a halogenated hydrocarbon such as dichloromethane at a low temperature eg. -30°C to ambient temperature, such as -20°C to 0°C, optionally in the presence of a base, eg. an organic base such as an amine, eg. triethylamine or a cyclic amine such as *N*-methylmorpholine.

The amine of general formula (III) may be prepared by coupling an acid of formula (IV), or an active derivative thereof

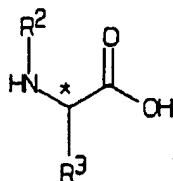
(IV)



with an amine of formula (V)

5

(V)



10

Followed by removal of any protecting groups.

Active derivatives of acids of formula (IV) include for example acid anhydrides or acid halides such as acid chlorides as outlined earlier.

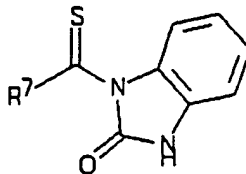
Amino acids and their derivatives as depicted by general formulae (IV) and (V) can be obtained in the optically pure or racemic form. In the homochiral form they provide asymmetric building blocks for the enantiospecific synthesis of compounds of general formula (I). Many of these derivatives can be readily obtained from commercially available starting materials using methods known to those skilled in the art. (See "The Practice of Peptide Synthesis" by M. Bodanszky *et al*, Springer Verlag, New York, 1984 and P.L. Durette, WO92/213460).

20

In cases where Y is C=S, intermediates of general formula (I) may be prepared by reacting a thioacyl compound of formula (VI)

25

(VI)



30

Wherein R⁷ is defined above, with an amine of formula (III)

The thioamidation reaction may be achieved simply by reaction of (III) and (VI) in a suitable solvent, for example an inert organic solvent such as an ether, eg. a cyclic ether such as tetrahydrofuran, an amide eg. a substituted amide such as

dimethylformamide, or a halogenated hydrocarbon such as dichloromethane at a low temperature eg. -30°C to ambient temperature, such as -20°C to 0°C.

Thioacyl derivatives of formula (VI) may be prepared according to a literature procedure (B. Zacharie *et al.*, *Bioorg. Med. Chem. Letters*, 1993, 3(4), 619-24).

Compounds of formula (I) may also be prepared by interconversion of other compounds of formula (I). Thus, for example, a compound of formula (I) wherein R¹ is a C₁₋₆ alkyl group may be prepared by hydrogenation (using palladium on carbon in suitable solvent, such as an alcohol - eg ethanol) of a compound of formula (I) wherein R¹ is a C₂₋₆ alkenyl group.

Any mixtures of final products or intermediates obtained can be separated on the basis of the physico-chemical differences of the constituents, in known manner, into the pure final products or intermediates, for example by chromatography, distillation, fractional crystallization, or by formation of a salt if appropriate or possible under the circumstances.

Compounds according to the invention exhibit *in vitro* inhibition of TNF release by cells. The compounds according to the invention may also exhibit *in vitro* inhibiting activities with respect to matrix metalloproteinases such as stromelysin, collagenase and gelatinase. The activity and selectivity of the compounds may be determined by use of the appropriate enzyme inhibition test, for example as described in Example A hereinafter.

This invention also relates to a method of treatment for patients (including man and/or mammalian animals raised in the dairy, meat or fur industries or as pets) suffering from disorders or diseases which can be attributed to stromelysin as previously described, and more specifically, a method of treatment involving the administration of the matrix metalloproteinase inhibitors of formula (I) as the active constituents.

Accordingly, the compounds of formula (I) can be used among other things in the treatment of osteoarthritis and rheumatoid arthritis, and in diseases and indications resulting from the over-expression of these matrix metalloproteinases such as found in certain metastatic tumour cell lines.

As mentioned above, compounds of formula (I) are useful in human or veterinary medicine since they are active as inhibitors of TNF and/or MMPs. Accordingly in another aspect, this invention concerns:

1
/ a method of management (by which is meant treatment of prophylaxis) of
Y disease or conditions mediated by TNF and/or MMPs in mammals, in particular in
humans, which method comprises administering to the mammal an effective amount
of a compound of formula (I) above, or a pharmaceutically acceptable salt thereof;

5 and

a compound of formula (I) for use in human or veterinary medicine,
particularly in the management (by which is meant treatment or prophylaxis) of
diseases or conditions mediated by TNF and/or MMPs; and

the use of a compound of formula (I) in the preparation of an agent for the
10 management (by which is meant treatment or prophylaxis) of diseases or conditions
mediated by TNF and/or MMPs.

The disease or conditions referred to above include inflammatory diseases,
autoimmune diseases cancer, cardiovascular diseases, diseases involving tissue
breakdown such as rheumatoid arthritis, osteoarthritis, osteoporosis,
15 neurodegeneration, Alzheimer's disease, atherosclerosis, congestive heart failure,
stroke, vasculitis, Crohn's disease, ulcerative colitis, multiple sclerosis, periodontitis,
gingivitis and those involving tissue breakdown such as bone resorption,
haemorrhage, coagulation, acute phase response, cachexia and anorexia, acute
infections, HIV infections, fever, shock states, graft versus host reactions,
20 dermatological conditions, surgical wound healing, psoriasis, atopic dermatitis,
epidermolysis bullosa, tumour growth, angiogenesis and invasion by secondary
metastases, ophthalmological disease, retinopathy, corneal ulceration, reperfusion
injury, migraine, meningitis, asthma, rhinitis, allergic conjunctivitis, eczema and
anaphylaxis.

25 For the treatment of rheumatoid arthritis, osteoarthritis, and in diseases and
indications resulting from the over-expression of matrix metalloendoproteinases such
as found in certain metastatic tumour cell lines or other diseases mediated by the
matrix metalloendoproteinases or increased TNF production, the compounds of
formula (I) may be administered orally, topically, parenterally, by inhalation spray
30 or rectally in dosage unit formulations containing non-toxic pharmaceutically
acceptable carriers, adjuvants and vehicles. The term parenteral as used herein
includes subcutaneous injections, intravenous, intramuscular, intrasternal injection
or infusion techniques. In addition to the treatment of warm-blooded animals such

as mice, rats, horses, cattle, sheep, dogs, cats etc, the compounds of the invention are effective in the treatment of humans.

The pharmaceutical composition containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the techniques described in the US Patents 4,256,108; 4,166,452; and 4,265,874 to form osmotic therapeutic tablets for control release.

Formulations for oral use may also be presented as hard gelatin capsules where in the active ingredient is mixed with an inert solid diluent, for example calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids,

for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such a polyoxyethylene with partial esters derived from fatty acids and
5 hexitol anhydrides, for example polyoxyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate, one or more colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredient in
10 a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-
15 oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified, for
20 example sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum
25 acacia or gum tragacanth, naturally-occurring phosphatides, for example soya bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavouring agents.

30 Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavouring and colouring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known

art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be in a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable
5 vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

10 The compounds of formula (I) may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene
15 glycols.

For topical use, creams, ointments, jellies, solutions or suspensions, etc. containing the compounds of Formula (I) are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

Dosage levels of the order of from about 0.05 mg to about 140 mg per
20 kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 2.5 mg to about 7 gms per patient per day). for example, inflammation may be effectively treated by the administration of from about 0.01 to 50 mg of the compound per kilogram of body weight per day (about 0.5 mg to about 3.5 gms per patient per day).

25 The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may vary from about 5 to about 95 percent of the total composition. Dosage unit forms will generally contain between from about 1
30 mg to about 500 mg of an active ingredient.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the

administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

The following Examples are intended to illustrate the preparation of compounds of Formula (1), and as such are not intended to limit the invention as set forth in the claims.

In the Examples, the following abbreviations are used:

RT	Room temperature
TNF α	Tumour necrosis factor α
10 ELISA	Enzyme linked immunosorbent assay
LPS	Lipopolysaccharide (Endotoxin)
EDC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
<u>Intermediate 1</u>	Potassium 4-phthalimidobutanesulphonate

A solution of 1,4-butanedisulfoxide (10g, 73.4 mmol) in dry 1,4-dioxane (6ml) was added dropwise at 60°C to a stirred suspension of potassium phthalimide (13.6g, 73.4 mmol) in dry dimethylformamide (75ml). The mixture was then heated at 110°C for 1h, cooled to <5°C and diluted with tetrahydrofuran (100ml). The product was removed by filtration, washed with ether (2x100ml) and dried *in vacuo* to provide the title compound (20.1g, 85%) as a white solid.

Similarly prepared was:

Intermediate 2 Potassium 3-phthalimidopropanesulphonate

From 1,4-propanedisulfoxide, as a white solid (37g, 87%)

Intermediate 3 4-Phthalimidobutanesulphonyl chloride

Phosphorus pentachloride (6.48g, 31.1 mmol) was added portionwise at RT to a stirred suspension of potassium 4-phthalimidobutanesulphonate (10g, 31.1 mmol) in phosphoryl chloride (30ml). The exothermic reaction was allowed to cool to RT over 0.5h and excess phosphoryl chloride removed by distillation at atmospheric pressure. The cooled mixture was then diluted with dichloromethane (50ml) and precipitated potassium chloride removed by filtration through hyflo. The filtrate was evaporated to dryness *in vacuo* and the residue triturated with carbon tetrachloride (30ml) to precipitate the product, as a pale pink solid (7.4g, 79%).

Tlc: EtOAc, R_f 0.71

Similarly prepared was:

Intermediate 4 4-Phthalimidopropanesulphonyl chloride

From intermediate 2, as a white solid (11g, 65%)

Tlc: EtOAc, R_f 0.67

Intermediate 5 5-Phthalimidopentanoic acid

5 A solution of 5-aminopentanoic acid (9.26g, 79.1 mmol) and sodium carbonate (8.45g, 79.85 mmol) in water (300 ml) was treated with *N*-carboethoxyphthalimide (17.2g, 78.6 mmol) and the mixture stirred at room temperature for 4h. The solution was acidified to pH1 using 6M hydrochloric acid and the precipitated product isolated as a white solid (13.5g, 69%).

Tlc: EtOAc/Hexane/HOAc (1:1:0.1), R_f 0.33

10 Similarly prepared were:

Intermediate 6 3-Phthalimidopropanoic acid

From 2-aminopropanoic acid, as a white solid (7.5 g, 61%).

Tlc: EtOAc/Hexane/HOAc (1:1:0.1), R_f 0.28

Intermediate 7 4-Phthalimidobutanoic acid

15 From 4-aminobutanoic acid, as a white solid (7.4 g, 63%).

Tlc: EtOAc/Hexane/HOAc (1:1:0.1), R_f 0.30

Intermediate 8 6-Phthalimidohexanoic acid

From 6-aminohexanoic acid, as a white solid (5.8g, 58%).

Tlc: EtOAc/Hexane/HOAc (1:1:0.1), R_f 0.36

20 **Intermediate 9** 2-Phenylacetamidoaniline

Phenylacetyl chloride (8.06 g) in dichloromethane (20 ml) was added dropwise to a solution of *o*-phenylene diamine (5.63 g) in dichloromethane (100 ml) and triethylamine (6.3 g) at 0°C. The mixture was stirred for 18 h and the suspension washed with water and brine. The solution was dried (Na_2SO_4) and
25 evaporated. The solid residue was purified by flash column chromatography (CH_2Cl_2 /ether (3:1)) to give the title compound as colourless solid (1.32 g).

TLC R_f 0.23 (CH_2Cl_2 / ether (3:1))

Intermediate 10 2-(2-Phenyl-1-thioethylamino)aniline

30 A mixture of sodium carbonate (0.95 g) and phosphorus pentasulphide (3.9 g) in dry tetrahydrofuran (30 ml) was stirred at RT for 30 min, then cooled to 0°C and a solution of intermediate 9 (0.23 g) in tetrahydrofuran (10 ml) added. The yellow mixture was stirred at RT for 3 h, then a solution of sodium phosphate (10% aq, 50 ml) added. The mixture was stirred for 10 min, then 2:1 ethyl acetate/hexanes (30 ml) was added and the mixture stirred vigorously for 10 min.

The phases were separated and the organic solvent was washed with brine, dried and evaporated. The gummy residue was purified by flash column chromatography (ether) to give the title compound (36 mg) as a colourless solid.

TLC R_f 0.65 (ether).

5 Intermediate 11 1-(2-Phenyl-1-thioethyl)benzimidazolone

Carbonylditriazole (49 mg) was added to a solution of intermediate 10 (36 mg) in tetrahydrofuran (10 ml) at 0°C. The solution was stirred for 2 h, then evaporated to give a pale yellow solid. This was purified by flash column chromatography (2:1 ether/hexanes) to give the title compound as pale yellow solid (28.3 mg).

TLC R_f 0.45 (2:1 ether/hexanes).

Example 1 5-Phthalimidopentanoyl-L-leucyl-L-phenylalanine *N*-methyl amide

A solution of intermediate 5 (186 mg, 0.75 mmol), L-leucyl-L-phenylalanine *N*-methylamide (200 mg, 0.69 mmol) and 1-hydroxybenzotriazole (110 mg, 0.82 mmol) in dry tetrahydrofuran (20 ml) was treated at 0°C with EDC (104 mg, 0.77 mmol). The mixture was allowed to warm to RT and stirred overnight. The solvent was removed *in vacuo* and the residue dissolved in dichloromethane (50 ml). The solution was then washed sequentially with 2M hydrochloric acid (2x50 ml), 8% sodium bicarbonate (2x50 ml), water (50 ml) and brine (50 ml), dried (MgSO₄) and evaporated *in vacuo* to provide the title compound as a white solid (180 mg, 50%).

Tlc: EtOAc, R_f 0.42

MH⁺ 521, MNa⁺ 543

Similarly prepared were:

Example 2 2-Phthalimidoacetyl-L-leucyl-L-phenylalanine *N*-methylamide

25 From *N*-phthalimidoglycine, as a white solid (220 mg, 67%).

Tlc: EtOAc, R_f 0.31

MH⁺ 479, MNa⁺ 501

Example 3 3-Phthalimidopropanoyl-L-leucyl-L-phenylalanine *N*-methylamide

From intermediate 6, as a white solid (220 mg, 65%).

30 Tlc: EtOAc, R_f 0.21

MH⁺ 493, MNa⁺ 515

Example 4 4-Phthalimidobutanoyl-L-leucyl-L-phenylalanine *N*-methylamide

From intermediate 7, as a white solid (220 mg, 56%).

Tlc: EtOAc, R_f 0.45

MH⁺ 507, MNa⁺ 529

Example 5 6-Phthalimidohexanoyl-L-leucyl-L-phenylalanine *N*-methyl amide

From intermediate 8, as a white solid (240 mg, 65%).

Tlc: EtOAc, R_f 0.37

5 MH⁺ 535, MNa⁺ 557

Example 6 2-Thiophenecarboxyl-L-leucyl-L-phenylalanine *N*-methylamide

From commercially available thiophene-2-carboxylic acid, as a white solid (399 mg, 97%).

Tlc: MeOH/CH₂Cl₂ (5:95) R_f 0.43

10 **Example 7** Propanoyl-L-leucyl-L-phenylalanine *N*-methylamide

From commercially available propanoyl chloride, as a white solid (200 mg, 84%).

Tlc: MeOH/CH₂Cl₂ (5:95), R_f 0.31

MH⁺ 348, MNa⁺ 370

15 **Example 8** 4-Oxopentanoyl-L-leucyl-L-phenylalanine *N*-methyl amide

From commercially available levulinic acid, as a white solid (650 mg, 97%).

Tlc: MeOH/CH₂Cl₂ (5:95), R_f 0.42

MH⁺ 490, MNa⁺ 512

Example 9 5-Phthalimidopentanoyl-L-valinyl-L-phenylalanine *N*-methylamide

20 From intermediate 5 and L-valinyl-L-phenylalanine *N*-methylamide, as a white solid (140 mg, 40%).

Tlc: EtOAc, R_f 0.41

MH⁺ 491, MNa⁺ 513

Example 10 2-(5-Imidazo)acetyl-L-leucyl-L-phenylalanine *N*-methylamide

25 From commercially available imidazole-5-acetic acid and L-leucyl-L-phenylalanine *N*-methylamide, as a white solid (33 mg, 8%).

Tlc: 2% Ammonia-10% MeOH-CH₂Cl₂, R_f 0.18

Example 11 3-(5-Imidazo)propanoyl-L-(*S*-methyl)cysteinyl-L-phenylalanine *N*-methylamide

30 From commercially available imidazole-5-propionic acid and L-(*S*-methyl)cysteinyl-L-phenylalanine *N*-methylamide, as a white solid (20 mg, 4%).

Tlc: 2% Ammonia-10% MeOH-CH₂Cl₂, R_f 0.15

Example 12 2-Methoxy-2-oxoacetyl-L-leucyl-L-phenylalanine *N*-methylamide

From methyl oxalyl chloride and L-leucyl-L-phenylalanine *N*-methanamide, as a white solid (300 mg, 46%).

Tlc: 5% MeOH-CH₂Cl₂, R_f 0.30

Example 13 3-Methoxy-3-oxoacetyl-L-leucyl-L-phenylalanine *N*-methanamide

5 From methyl malonyl chloride and L-leucyl-L-phenylalanine *N*-methanamide, as a white solid (120 mg, 72%).

Tlc: 5% MeOH-CH₂Cl₂, R_f 0.35

Example 14 2-Oxalyl-L-leucyl-L-phenylalanine *N*-methanamide

A solution of example 12 (200mg) in methanol (5ml) was treated at RT with
10 a solution of lithium hydroxide (51mg) in water (5ml). The mixture was stirred at RT for 4 h, then concentrated *in vacuo*. The residue was then acidified with 1N hydrochloric acid, the precipitated product removed by filtration and dried *in vacuo* to provide the title compound (141 mg, 74%) as a white solid.

Tlc: 5% MeOH-CH₂Cl₂, R_f 0.05

15 Similarly prepared was:

Example 15 3-Malonyl-L-leucyl-L-phenylalanine *N*-methanamide

From example 13, as a white solid (80 mg, 82%).

Tlc: 5% MeOH-CH₂Cl₂, R_f 0.08

Example 16 *N*-(2-Phenyl-1-thioethyl)-L-leucyl-L-phenylalanine *N*-methanamide

20 A solution of L-leucyl-L-phenylalanine *N*-methanamide (45 mg) and intermediate 11 (28.3 mg) in tetrahydrofuran was stirred at 0°C to RT overnight. The solvent was evaporated and the residue partitioned between CH₂Cl₂ and 1N HCl. The organic phase was washed with brine, dried and evaporated to give the title compound as colourless solid (58.5 mg).

25 TLC R_f 0.35 (ether).

Example 17 4-Phthalimidobutanesulphonyl-L-leucyl-L-phenylalanine *N*-methanamide

A solution of intermediate 3 (513mg, 1.7 mmol) in dry dichloromethane (2ml) was added at 0°C to a stirred solution of L-leucyl-L-phenylalanine-*N*-
30 methanamide (500 mg, 1.7 mmol) and triethylamine (355 µl, 2.6 mmol) in dry dichloromethane (20ml). The mixture was allowed to warm to RT and stirred overnight. The mixture was diluted with dichloromethane (50ml) then washed sequentially with 2M hydrochloric acid (2x50ml), 8% sodium bicarbonate (2x50ml), water (50ml) and brine (50ml), dried (MgSO₄) and evaporated *in vacuo* to provide

a yellow foam. Purification by column chromatography eluting with 5% methanol in dichloromethane provided the product as a white solid (370 mg, 39%).

Tlc: MeOH/CH₂Cl₂ (1:10), R_f 0.47

MH⁺ 557, MNa⁺ 579

5 Similarly prepared were:

Example 18 Methanesulphonyl-L-leucyl-L-phenylalanine *N*-methylamide

From methanesulphonyl chloride, as a white solid (309mg, 75%)

Tlc: MeOH/CH₂Cl₂ (5:95) R_f 0.26

MH⁺ 370, MNa⁺ 392, [2M+Na]⁺ 739

10 **Example 19** Ethanesulphonyl-L-leucyl-L-phenylalanine *N*-methylamide

From ethanesulphonyl chloride, as a white solid (160 mg, 61%).

Tlc: EtOAc, R_f 0.71

MH⁺ 384, MNa⁺ 406

Example 20 Butanesulphonyl-L-leucyl-L-phenylalanine *N*-methylamide

15 From butanesulphonyl chloride, as a white solid (180 mg, 64%).

Tlc: EtOAc, R_f 0.64

MH⁺ 412, MNa⁺ 434

Example 21 Phenylsulphonyl-L-leucyl-L-phenylalanine *N*-methylamide

From benzenesulphonyl chloride, as a white solid (180 mg, 61%).

20 Tlc: EtOAc, R_f 0.67

MH⁺ 432, MNa⁺ 454

Example 22 Phenylmethanesulphonyl-L-leucyl-L-phenylalanine *N*-methylamide

From benzylsulphonyl chloride, as a white solid (210 mg, 67%).

Tlc: EtOAc, R_f 0.52

25 MH⁺ 446, MNa⁺ 468

Example 23 Ethenylsulphonyl-L-leucyl-L-phenylalanine *N*-methylamide

From 2-chloroethylsulphonyl chloride, as a white solid (309 mg; 75%).

Tlc: MeOH/CH₂Cl₂, R_f 0.31

Example 24 4-Phthalimidobutanesulphonyl-L-valinyl-L-phenylalanine *N*-methylamide

30

From intermediate 3 and L-valinyl-L-phenylalanine *N*-methylamide, as a white solid (3.5 g, 76%).

Tlc: EtOAc, R_f 0.37

MH⁺ 543, MNa⁺ 565

Example 25 3-Phthalimidopropanesulphonyl-L-leucyl-L-phenylalanine *N*-methylamide

From intermediate 4 and L-leucyl-L-phenylalanine *N*-methylamide, as a white solid (210 mg, 48%).

5 Tlc: EtOAc, R_f 0.42

MH⁺ 543, MNa⁺ 565

Example 26 *N*-(4-Aminobutylsulfonyl)-L-leucyl-L-phenylalanine *N*-methylamide

Hydrazine hydrate (56%, 18.2 ml) was added to a solution of example 17 (7.1 g) in ethanol (120 ml) and the mixture was heated at reflux for 30 min. The mixture was then cooled to RT and diluted with ethanol (300 ml), chloroform (100 ml) and water (200 ml). The layers were separated and the organic layer was dried (Na₂SO₄) and evaporated *in vacuo*. The residue was then purified by flash column chromatography, eluting with 0-10% methanol in chloroform containing 2.5% triethylamine, to give the title compound (5.39 g, 99%) as a colourless solid.

15 TLC R_f 0.03 (2.5% Et₃N-10% MeOH-CH₂Cl₂)

Example 27 *N*-(4-Acetylaminobutylsulfonyl)-L-leucyl-L-phenylalanine *N*-methylamide

Acetic anhydride (1.0 ml) was added to a solution of Example 26 (0.50 g) and triethylamine (0.030 ml) in dichloromethane (15 ml), and the mixture was stirred at RT 18 h. The mixture was then diluted with dichloromethane (20 ml), washed with water (20 ml) and dried (Na₂SO₄). The solvent was removed *in vacuo* and the residue was crystallised from methanol-ethyl acetate to give the title compound (0.438 g, 80%) as a colourless solid.

TLC R_f 0.31 (10% MeOH-CH₂Cl₂)

25 Similarly prepared were:

Example 28 *N*-(4-Benzoylaminobutylsulfonyl)-L-leucyl-L-phenylalanine *N*-methylamide

From benzoyl chloride (0.20 ml) and Example 26 (0.621 g), to give the title compound (0.690 g, 89%) as a colourless solid.

30 TLC R_f 0.30 (10% MeOH-CH₂Cl₂)

Example 29 *N*-(4-Methanesulfonylaminobutylsulfonyl)-L-leucyl-L-phenylalanine *N*-methylamide

From methanesulfonyl chloride (1.68 ml) and example 26 (0.620 g) to give the title compound (0.620 g, 79%) as a colourless solid.

TLC R_f 0.35 (10% MeOH-CH₂Cl₂)

Example 30 *N*-(4-(Ethylcarbamoylamino)butylsulfonyl)-L-leucyl-L-phenylalanine
N-methanamide

From ethyl isocyanate (0.167 g) and example 26 (1.00 g), to give the title
5 compound (0.860 g, 76%) as a colourless solid.

TLC R_f 0.19 (10% MeOH-CH₂Cl₂)

Example 31 *N*-[4-(Pyrrolidine-2,5-dione-1-yl)butylsulfonyl]-L-leucyl-L-
phenylalanine *N*-methanamide

Succinic anhydride (0.261 g) was added to a solution of example 26 (0.80 g)
10 in dichloromethane (20 ml), and the mixture was stirred 2 h at RT. The mixture was
diluted with tetrahydrofuran (20 ml) and stirring was continued 18 h. At this time,
a solution of EDC (0.395 g) and *N*-hydroxybenzotriazole (0.304 g) in
tetrahydrofuran/dichloromethane (4:1, 25 ml) was added and stirring continued for
24 h. The solvent was removed *in vacuo* and the residue was partitioned between
15 dichloromethane (100 ml) and 1N hydrochloric acid. The layers were separated and
the organic layer was washed with water (100 ml), sat. sodium bicarbonate (100 ml),
and brine (100 ml), then dried (Na₂SO₄) and evaporated, *in vacuo* to a solid residue.
Purification by column chromatography, eluting with 2% methanol in
dichloromethane provided the title compound (0.304 g, 31%) as a colourless solid.

20 TLC R_f 0.29 (10% MeOH-CH₂Cl₂)

Example A

Collagenase inhibition activity

The potency of compounds of general formula (I) to act as inhibitors of
collagenase was determined by the procedure of Cawston and Barrett (Anal.
25 Biochem., 99:340-345, 1979) whereby a 1mM solution of the inhibitor being tested
or dilutions thereof was incubated at 37°C for 16 hours with collagen and collagenase
(buffered with 50 mM Tris, pH 7.6 containing 5 mM CaCl₂, 0.05% Brij 35, 60 mM
NaCl and 0.02% NaN₃). The collagen was acetylated ³H or ¹⁴C-collagen prepared
by the method of Cawston and Murphy (Methods in Enzymology, 80:711, 1981).
30 The choice of radiolabel did not alter the ability of collagenase to degrade the
collagen substrate. The samples were centrifuged to sediment undigested collagen
and an aliquot of the radioactive supernatant removed for assay on a scintillation
counter as a measure of hydrolysis. The collagenase activity in the presence of 1mM
inhibitor, or a dilution thereof, was compared to activity in a control devoid of

inhibitor and the results reported as that inhibitor concentration effecting 50% inhibition of the collagenase (IC_{50}).

Example B

Stromelysin inhibition activity

5 The potency of compounds of general formula (I) to act as inhibitors of stromelysin was determined using the procedure of Nagase et al (Methods in Enzymology Vol 254, 1994), whereby a 0.1 mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with stromelysin and 3H transferrin (buffered with 50 mM Tris, pH 7.6 containing 10 mM $CaCl_2$, 150M
10 NaCl, 0.05% Brij, 35, and 0.02% NaN_3). The transferrin was carboxymethylated with 3H iodoacetic acid. The stromelysin activity in the presence of 1 mM, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the results reported as that inhibitor concentration effecting 50% inhibition of the stromelysin (IC_{50}).

15 Example C

Gelatinase inhibition activity

 The potency of the compounds of general formula (I) to act as inhibitors of gelatinase was determined using the procedure of Harris & Krane (Biochem Biophys. Acta, 258:566 - 576, 1972), whereby a 1 mM solution of the inhibitor being tested
20 or dilutions thereof was incubated at 37°C for 16 hours with gelatinase and heat denatured 3H or ^{14}C -acetylated collagen (buffered with 50 mM Tris, pH 7.6 containing 5 mM $CaCl_2$, 0.05% Brij-35 and 0.02% NaN_3). The 3H or ^{14}C gelatin was prepared by denaturing 3H or ^{14}C -collagen produced according to the method of Cawston and Murphy (Methods in Enzymology, 80:711, 1981) by incubation at 60°C
25 for 30 minutes. Undigested gelatin was precipitated by addition of trichloroacetic acid and centrifugation. The gelatinase activity in the presence of 1 mM, or dilution thereof, was compared to the activity in a control devoid of inhibitor and results reported as that inhibitor concentration effecting 50% inhibition of the gelatinase (IC_{50}).

30 Example D

MMP Inhibition Activity-Fluorimetric Assay

 The potency of compounds of general formula (I) to act as inhibitors of collagenase-1(MMP-1), collagenase-2 (MMP-8), gelatinase-A (MMP-2), gelatinase-B (MMP-9) and stromelysin-1(MMP-3) was determined using the following procedure:

Inhibitors were dissolved in dimethylsulphoxide containing 0.02% β -mercaptoethanol and serial dilutions are prepared. Activated enzyme was incubated in assay buffer containing 50mM Tris, pH 7.4, 5mM CaCl_2 , 0.002% NaN_3 and Brij 35 in the presence and absence of inhibitor. Samples were preincubated at 37°C for 15 minutes before the addition of the fluorimetric substrate (Mca-Pro-Leu-Dpa-Ala-Arg-NH₂) to a final concentration of 10mM. The assay was incubated for 90 minutes at 37°C and then read in a Fluoroscan II at λ_{ex} (355nm) and λ_{em} (460nm). The enzyme activity was compared to activity in a control devoid of inhibitor and the results reported as that inhibitor concentration effecting 50% inhibition of the stromelysin (IC_{50}).

Example E

Inhibition of $\text{TNF}\alpha$ production

The potency of the compounds of general formula (I) to act as inhibitors of the production of $\text{TNF}\alpha$ was determined using the following procedure. A 1mM solution of the inhibitor being tested or dilutions thereof was incubated at 37° C in an atmosphere of 5% CO_2 with THP-1 cells (human monocytes) suspended in RPMI 1640 medium and 20 μM β -mercaptoethanol at a cell density of $1 \times 10^6/\text{ml}$ and stimulated with 5 $\mu\text{g}/\text{ml}$ final concentration of LPS. After 18 hours the supernatant was assayed for the levels of $\text{TNF}\alpha$ using a commercially available ELISA kit (R & D Systems). The activity in the presence of 0.1mM inhibitor or dilutions thereof was compared to activity in a control devoid of inhibitor and results reported as that inhibitor concentration effecting 50% inhibition of the production of $\text{TNF}\alpha$.

Example F

Adjuvant arthritic rat model

Compounds of general formula (I) were evaluated in an adjuvant arthritis model in the rat based on the methods employed by B.B. Newbould (1963), Br.J.Pharmacol, 21, 127-136 and C.M. Pearson and F.D. Wood (1959), Arthritis Rheum, 2, 440-459. Briefly, male Wistar rats (180-200g) were injected at the base of the tail with Freund's adjuvant. Twelve days later the responding animals were randomised into experimental groups. Compounds of general formula (I) were dosed either orally as a suspension in 1% methyl cellulose or intraperitoneally in 0.2% carboxymethylcellulose from day 12 to the end of the experiment on day 22. Hind paw volumes were measured every two days from day 12 onwards and X-rays were

taken of the hind feet on completion of the experiment. Results were expressed as the percent increase of foot volume over day 12 values.

Example G

Mouse ovarian carcinoma xenograft model

5 Compounds of general formula (I) were evaluated in an ovarian carcinoma xenograft model of cancer, based on that described by B. Davies et al (1993), Cancer Research, 53, 2087-2091. This model, in brief, consists of inoculating female nu/nu mice with 1×10^9 OVCAR3-icr cells into the peritoneal cavity. Compounds of general formula (I) are administered by the oral route as a suspension in 1% methyl cellulose or intraperitoneally as a suspension in phosphate buffered saline in 0.01% Tween-20. At the conclusion of the experiment (4-5 weeks) the number of peritoneal cells are counted and any solid tumour deposits weighed. In some experiments tumour development is monitored by measurement of tumour specific antigens.

Example H

Rat mammary carcinoma model

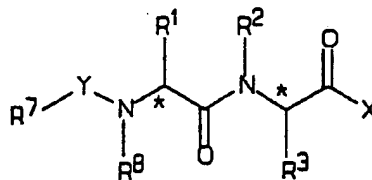
 Compounds of general formula (I) were evaluated in a HOSP.1 rat mammary carcinoma model of cancer (S.Eccles et al (1995), Cancer Research, in press). This model consists of the intravenous inoculation of female CBH/cbi rats with 2×10^4 tumour cells into the jugular vein. Compounds of general formula (I) are administered by the oral route as a suspension in 1% methyl cellulose or intraperitoneally as a suspension in phosphate buffered saline in 0.01% Tween-20. At the conclusion of the experiment (4-5 weeks) the animals were killed, the lungs were removed and individual tumours counted after 20 hours fixation in Methacarn.

CLAIMS

1. Compounds of formula (I)

5

(I)



10

wherein:

Y is C=O, C=S or S(O)₁₋₂;

15 R¹ is a C₁₋₆ alkyl, C₂₋₆ alkenyl, C₁₋₆ alkyl-aryl, aryl, C₁₋₆ alkyl-heteroaryl, heteroaryl or C₁₋₆ alkyl-AR⁹ group where A is O, NR⁹ or S(O)_m where m=0-2, and R⁹ is H, C₁₋₄ alkyl, aryl, heteroaryl, C₁₋₄ alkyl-aryl or C₁₋₄ alkyl-heteroaryl; if A=NR⁹ the R⁹ groups may be the same or different;

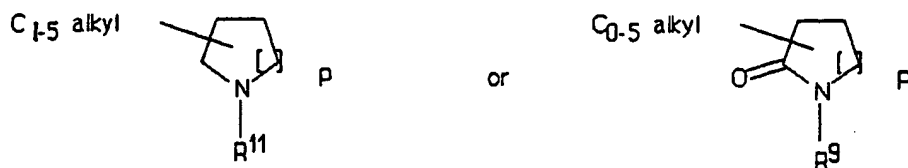
R² is hydrogen or C₁₋₆ alkyl;R³ is [Alk]_nR⁶ where Alk is C₁₋₆ alkyl or C₂₋₆ alkenyl and n is zero or 1;

20 X is NR⁴R⁵ where R⁴ is hydrogen, aryl, heteroaryl, C₁₋₆ alkyl-heteroaryl, cyclo(C₃₋₆)alkyl, C₁₋₆alkyl-cyclo(C₃₋₆)alkyl, heterocyclo(C₄₋₆)alkyl (such as pyrrolidine or piperidine) or C₁₋₆ alkyl-heterocyclo(C₄₋₆)alkyl or the group C₁₋₆ alkyl optionally substituted by amino (NH₂), aryl, arylamino, protected amino, di(C₁₋₆ alkyl)amino, mono(C₁₋₆ alkyl)amino, CO₂H, protected carboxyl, carbamoyl, mono(C₁₋₆ alkyl) carbamoyl, di (C₁₋₆ alkyl) carbamoyl, and R⁵ is hydrogen, or NR⁴R⁵ forms a ring such as pyrrolidino, piperidino or morpholino;

25 R⁷ is aryl (optionally substituted with R¹⁰), heteroaryl (optionally substituted with R¹⁰), C_{1,5} alkyl (optionally substituted with R¹⁰), C_{1,5} alkyl-aryl (optionally substituted with R¹⁰), C_{1,5} alkyl-heteroaryl (optionally substituted with R¹⁰), cyclo(C₃₋₆)alkyl (optionally substituted with R¹⁰), cyclo(C₃₋₆)alkenyl (optionally substituted with R¹⁰), C_{1,5} alkyl-cyclo(C₃₋₆)alkyl (optionally substituted with R¹⁰), the groups

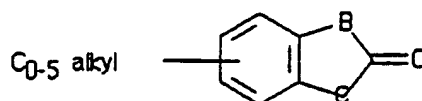
30

34



where $p=1-2$, or the group

5



10

where B and C are selected from the groups O, S, $C(R^9)_2$ and NR^9 and these may be the same or different;

15 when Y is $C=O$, R^7 may also be CO_2R^2 ;

R^8 is hydrogen;

R^6 is an optionally substituted cyclo(C_{3-6})alkyl, cyclo(C_{3-6})alkenyl, C_{1-6} alkyl, aryl, C_{1-6} alkoxy-aryl, benzyloxyaryl, heteroaryl, COR^9 , C_{1-3} alkyl-aryl, C_{1-3} alkyl-heteroaryl, C_{1-6} alkyl- CO_2R^9 , C_{1-6} alkyl- NHR^9 , AR^9 , CO_2R^2 , $CONHR^{12}$, $NHCOR^{12}$,

20 $NHCO_2R^{12}$, $NHSO_2R^{12}$, amidine or guanidine group;

R^{12} is C_{1-4} alkyl, C_{1-4} alkylaryl, C_{1-4} alkyl-heteroaryl, cyclo(C_{3-6})alkyl, C_{1-4} alkyl-cyclo(C_{3-6}) alkyl, C_{2-6} alkenyl, C_{2-6} alkenyl-aryl, aryl or heteroaryl as defined above;

R^{10} is AR^9 , COR^{13} , SO_2R^9 (where R^9 is not H), $SO_2N(R^9)_2$, NR^9R^{11} , COR^9 , phthalimido or succinimido;

25 R^{11} is COR^9 , CO_2R^2 (where R^2 is not H), $CONHR^9$ or SO_2R^9 (where R^9 is not H); and

R^{13} is OH, OC_{1-4} alkyl, $O(C_{1-4}$ alkyl)aryl or $N(R^9)_2$ (in which R^9 is the same or different);

and the salts, solvates and hydrates thereof.

30 2. A compound of claim 1, where Y is $C=O$ or $C=S$.

3. A compound of claim 1, where Y is SO or SO₂.
4. A compound of claim 3, where Y is SO₂.
5. A compound of claim 2 or claim 3, where R⁴ is H or optionally substituted alkyl or NR⁴, R⁵ is a ring, R⁶ is not amidine and R¹⁰ is not SOR⁹.
- 5 6. A compound of any preceding claim where R¹ is C₁₋₆ alkyl or C₁₋₄ alkyl-AR⁹ and R⁹ is H, C₁₋₄ alkyl, heteroaryl or aryl.
7. A compound of any preceding claim, where R² is H or C₁₋₄ alkyl.
8. A compound of any preceding claim, where Alk is C₁₋₄ alkyl and R⁶ is C₁₋₄ alkyl, C₁₋₃ alkyl-aryl, C₁₋₃ alkyl-heteroaryl, AR⁹, CO₂R², CONHR¹², NHCOR¹²,
10 NHCO₂R¹², NHSO₂R¹², amidine or guanidine.
9. A compound of any preceding claim, where R⁵ is H.
10. A compound of claim 9, where R⁴ is H, C₁₋₆ alkyl, aryl, heteroaryl, C₁₋₂ alkyl-aryl or C₁₋₂ alkyl-heteroaryl.
11. A compound of any of claims 1 to 8, where NR⁴R⁵ is a 5-7 membered ring.
- 15 12. A compound of claim 11, wherein NR⁴R⁵ is pyrrolidine, piperidine or morpholine.
13. A compound of any preceding claim, where R⁷ is aryl (optionally substituted with R¹⁰), heteroaryl (optionally substituted with R¹⁰), C₁₋₄ alkyl-aryl (optionally substituted with R¹⁰), C₁₋₄ alkyl-heteroaryl (optionally substituted with R¹⁰), C₁₋₅ alkyl-R¹⁰, C₁₋₅ alkenyl-R¹⁰, cyclo(C₃₋₆)alkyl-R¹⁰ or, when Y is C=O, CO₂R².
20 14. A compound of any preceding claim, where R¹⁰ is COR¹³, NR⁹R¹¹, N(R⁹)₂, phthalimido or succinimido.
15. A compound of any preceding claim, where R¹¹ is COR⁹, CO₂R⁹ (provided R⁹ is not H), or SO₂R⁹ (provided R⁹ is not H).
- 25 16. A compound of any preceding claim, where R¹² is C₁₋₄ alkyl, C₁₋₄ alkylaryl or C₁₋₄ alkyl-heteroaryl.
17. A compound of any preceding claim, where R¹³ is OH, OC₁₋₄ alkyl or N(R⁹)₂.
18. A compound of claim 1, selected from
4-Phthalimidobutanesulphonyl-L-leucyl-L-phenylalanine *N*-methylethylamide
30 Methanesulphonyl-L-leucyl-L-phenylalanine *N*-methylethylamide
Ethanesulphonyl-L-leucyl-L-phenylalanine *N*-methylethylamide
Butanesulphonyl-L-leucyl-L-phenylalanine *N*-methylethylamide
Phenylsulphonyl-L-leucyl-L-phenylalanine *N*-methylethylamide
Phenylmethanesulphonyl-L-leucyl-L-phenylalanine *N*-methylethylamide

Ethenylsulphonyl-L-leucyl-L-phenylalanine *N*-methanamide

4-Phthalimidobutanesulphonyl-L-valinyl-L-phenylalanine *N*-methanamide

3-Phthalimidopropanesulphonyl-L-leucyl-L-phenylalanine *N*-methanamide.

19. A compound of claim 1, selected from

5 *N*-(4-Aminobutylsulfonyl)-L-leucyl-L-phenylalanine *N*-methanamide

N-(4-Acetylaminobutylsulfonyl)-L-leucyl-L-phenylalanine *N*-methanamide

N-(4-Benzoylaminobutylsulfonyl)-L-leucyl-L-phenylalanine *N*-methanamide

N-(4-Methanesulfonylaminobutylsulfonyl)-L-leucyl-L-phenylalanine

N-methanamide and

10 *N*-(4-(Ethylcarbamoylamino)butylsulfonyl)-L-leucyl-L-phenylalanine

N-methanamide.

20. A compound of claim 1, which is

N-[4-(Pyrrolidine-2,5-dion-1-yl)butylsulfonyl]-L-leucyl-L-phenylalanine *N*-methanamide.

15 21. A compound of claim 1, selected from

5-Phthalimidopentanoyl-L-leucyl-L-phenylalanine *N*-methanamide

2-Phthalimidoacetyl-L-leucyl-L-phenylalanine *N*-methanamide

3-Phthalimidopropanoyl-L-leucyl-L-phenylalanine *N*-methanamide

4-Phthalimidobutanoyl-L-leucyl-L-phenylalanine *N*-methanamide

20 6-Phthalimidohexanoyl-L-leucyl-L-phenylalanine *N*-methanamide

2-Thiophenecarboxyl-L-leucyl-L-phenylalanine *N*-methanamide

Propanoyl-L-leucyl-L-phenylalanine *N*-methanamide

4-Oxopentanoyl-L-leucyl-L-phenylalanine *N*-methanamide and

5-Phthalimidopentanoyl-L-valinyl-L-phenylalanine *N*-methanamide.

25 22. A compound of claim 1, selected from

2-(5-Imidazolo)acetyl-L-leucyl-L-phenylalanine *N*-methanamide

3-(5-Imidazolo)propanoyl-L-(*S*-methyl)cysteinyl-L-phenylalanine *N*-methanamide

2-Methoxy-2-oxoacetyl-L-leucyl-L-phenylalanine *N*-methanamide

30 3-Methoxy-3-oxopropanoyl-L-leucyl-L-phenylalanine *N*-methanamide

2-Oxalyl-L-leucyl-L-phenylalanine *N*-methanamide

3-Malonyl-L-leucyl-L-phenylalanine *N*-methanamide and

N-(2-Phenyl-1-thioethyl)-L-leucinyl-L-phenylalanine *N*-methanamide.

23. A compound of any preceding claim, in the form of a single enantiomer or diastereomer, or a mixture of such isomers.
24. A pharmaceutical composition for use in therapy, comprising a compound of any preceding claim, and a pharmaceutically-acceptable diluent or carrier.
- 5 25. Use of a compound of any of claims 1 to 23, for the manufacture of a human or veterinary medicament for the treatment or prevention of a condition associated with matrix metalloproteinases or that is mediated by $\text{TNF}\alpha$.
26. Use according to claim 25, wherein the condition is selected from cancer, inflammation and inflammatory diseases, tissue degeneration, periodontal disease, 10 ophthalmological disease, dermatological disorders, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infection, HIV infection, shock states, graft versus host reactions, autoimmune disease, reperfusion injury, meningitis, migraine.
27. Use according to claim 25, wherein the condition is selected from tumour 15 growth, angiogenesis, tumour invasion and spread, metastases, malignant ascites and malignant pleural effusion.
28. Use according to claim 25, wherein the condition is selected from rheumatoid arthritis, osteoarthritis, osteoporosis, asthma, multiple sclerosis, neurodegeneration, Alzheimer's, stroke, vasculitis, Crohn's disease and ulcerative 20 colitis.
29. Use according to claim 25, wherein the condition is selected from corneal ulceration, retinopathy and surgical wound healing.
30. Use according to claim 25, wherein the condition is selected from psoriasis, atopic dermatitis, chronic ulcers and epidermolysis bullosa.
- 25 31. Use according to claim 25, wherein the condition is selected from periodontitis and gingivitis.
32. Use according to claim 25, wherein the condition is selected from rhinitis, allergic conjunctivitis, eczema and anaphylaxis.
33. Use according to claim 25, wherein the condition is selected from 30 atherosclerosis and congestive heart failure.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 96/01136

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K5/023 C07K5/027 C07K5/03 C07K5/033 C07K5/062
 C07K5/083 A61K38/05 A61K38/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,95 06031 (IMMUNEX CORP) 2 March 1995 see claims	1,2, 5-17, 23-33
X	--- BIOCHEMISTRY (1990), 29(33), 7608-17 CODEN: BICHAW;ISSN: 0006-2960, 1990, XP002014430 BRADY, KENNETH ET AL: "Inhibition of chymotrypsin by peptidyl trifluoromethyl ketones: determinants of slow-binding kinetics" see page 7612, left-hand column, paragraph 8 - right-hand column, paragraph 1; table III --- -/--	1

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

27 September 1996

Date of mailing of the international search report

28.10.96

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Fuhr, C

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 96/01136

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US SCHELLENBERGER, VOLKER ET AL: "The specificity of chymotrypsin. A statistical analysis of hydrolysis data" XP002014431 see compounds 29701-42-6/RN and 65118-58-3/RN see abstract & EUR. J. BIOCHEM. (1991), 199(3), 623-36 CODEN: EJBCAI;ISSN: 0014-2956, 1991,</p> <p style="text-align: center;">---</p>	1
X	<p>DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US RIECHMANN, L. ET AL: "Kinetic studies on the mechanism and the specificity of peptide semisynthesis catalyzed by the serine proteases.alpha.-chymotrypsin and.beta.-trypsin" XP002014432 see compounds 29701-42-6/RN and 65118-58-3/RN see abstract & BIOCHEM. BIOPHYS. RES. COMMUN. (1984), 120(2), 686-91 CODEN: BBRC9;ISSN: 0006-291X, 1984,</p> <p style="text-align: center;">---</p>	1
X	<p>DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US PARADKAR, VIKRAM M. ET AL: "Aqueous-Like Activity of.alpha.-Chymotrypsin Dissolved in Nearly Anhydrous Organic Solvents" XP002014433 see compound 65118-58-3/RN see abstract & J. AM. CHEM. SOC. (1994), 116(11), 5009-10 CODEN: JACSAT;ISSN: 0002-7863, 1994,</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/GB 96/01136

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 99, no. 15, 10 October 1983 Columbus, Ohio, US; abstract no. 122898, EL-NAGGAR, A. M. ET AL: "Synthesis and biological activity of some new 2-(aminoacyl- and dipeptidyl-)aminopyrazine derivatives" XP002014535 see compound 87120-14-7 see abstract & FARMACO, ED. SCI. (1983), 38(6), 391-6 CODEN: FRPSAX;ISSN: 0430-0920, 1983,	1
X	--- CHEMICAL ABSTRACTS, vol. 116, no. 15, 13 April 1992 Columbus, Ohio, US; abstract no. 152346, EL-HADDAD, A. F. ET AL: "Synthesis of substituted 2-(aminoacyl)amino-6-halobenzothiazoles as possible antimicrobial agents" XP002014536 see compound 1394458-74-5 see abstract & ASIAN J. CHEM. (1992), 4(1), 144-51 CODEN: AJCHEW;ISSN: 0970-7077, 1992,	1
P,X	--- WO,A,95 13289 (CHIROSCIENCE LTD ;MONTANA JOHN (GB); DICKENS JONATHON (GB); OWEN D) 18 May 1995 see claims; examples	1,2, 5-17, 23-33
P,X	--- WO,A,96 11209 (CHIROSCIENCE LTD) 18 April 1996 see claims; examples	1,2, 5-17, 23-33
X	--- EP,A,0 394 989 (FUJISAWA PHARMACEUTICAL CO) 31 October 1990 see examples 6-(4), 30, 31 33, 34, 44-(4), 44-(5), 48-(1), 48-(2), 49-(1), 49-(2), 54-(2) see claims	1
A	--- WO,A,88 06890 (RES CORP TECHNOLOGIES INC) 22 September 1988 see claims; examples -----	1-33

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB96/01136

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-17, 23-33
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see continuation-sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB96/01136

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Reason: In view of the extremely large number of compounds falling under the claims 1-17 and 23-33, and of the absence of any technical support for these compounds in the description, the ISA considers that it is not economically reasonable to draw a search report covering the entire subject matter characterized above. The search has therefore been limited to the real examples given in the description and closely related ones and includes compounds having the alleged activities.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 96/01136

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9506031	02-03-95	AU-A- 7569494 EP-A- 0715619 FI-A- 960803 NO-A- 960723	21-03-95 12-06-96 22-04-96 23-02-96
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WO-A-9611209	18-04-96	AU-A- 3612795	02-05-96
EP-A-0394989	31-10-90	AT-T- 115961 CA-A- 2015359 DE-D- 69015244 DE-T- 69015244 JP-A- 3027399 US-A- 5164372	15-01-95 28-10-90 02-02-95 04-05-95 05-02-91 17-11-92
WO-A-8806890	22-09-88	AT-T- 106087 DE-D- 3889726 DE-T- 3889726 EP-A- 0358660 JP-T- 2502537	15-06-94 30-06-94 13-10-94 21-03-90 16-08-90